Prevention of Mold-Induced Softening in Air-Purged, Brined Cucumbers by Acidification

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ABSTRACT -

Mold-induced cucumber softening was prevented in air-purged fermentations by 0.16% acetic acid (equilibrated). Cucumber softening and pectate depolymerase activity increased in air-purged fermentations when the level of acid was decreased to 0.12% and below. Mold-induced softening was prevented in natural (not acidified) fermentations by delaying purging until indigenous microflora had reduced the brine pH to 4.0. Direct contact of air bubbles and cucumbers was not a requirement for subsurface mold growth. In air-purged commercial brines softening was evidenced by soft spots and skin blisters on cucumbers acidified with 0.05 and 0.0%; but not with 0.16%, acetic acid. In broth culture, growth of four mold isolates from soft cucumbers was inhibited by 0.3% acetic (pH 4.0) but not by up to 0.9% lactic acid (pH 3.0) at 5.3% NaCl.

INTRODUCTION

PURGING of CO₂ from fermenting brines is an effective means of preventing bloater damage in brined cucumbers (Etchells et al., 1973; Fleming et al., 1975; Costilow et al., 1977). Nitrogen has been recommended as the purging gas, but some commercial firms have been using air because of economic considerations. Fleming et al. (1975) and Gates and Costilow (1981) reported softening in laboratory-size, air-purged, naturally fermented brined cucumbers. Costilow et al. (1981) also reported minor softening near purger outlets in air-purged, commercial-size cucumber fermentations. Molds were found to cause softening in air-purged, natural fermentations of brined cucumbers (Costilow et al., 1980). In contrast, neither we nor Gates and Costilow (1981) have observed cucumber softening in "controlled fermentations" brined according to the procedure of Etchells et al. (1973), even when the fermentations were challenged with excessively high rates of air purging. Essential features of the controlled fermentation process, as initially described, include washing of the cucumbers, brine chlorination, acidification with acetic acid, buffering with sodium acetate, inoculation with Lactobacillus plantarum or Pediococcus cerevisiae or both, and N2 purging. The well-known antimicrobial action of acetic acid (Kirby et al., 1937; Levine and Fellers, 1940) was deemed a likely reason for failure of cucumbers to soften when controlled fermentations were air purged. The primary objective of this study was to determine the effects of acetic acid on cucumber softening in air-purged fermentations.

MATERIALS & METHODS

Laboratory fermentations

Unless otherwise stated, size no. 3 (3.8-5.1 cm diam), pickling cucumbers were obtained from local pickle companies or growers, and were stored at 10°C and ca 90% R.H. until use, within 3 days. Only cucumbers visibly free from damage and microbial spoilage were brined. Fermentations were in open, 5-gal, plastic pails or in

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1-gal or 1-qt glass jars at equilibrated concentrations of 5.3-6.6% NaCl and temperatures of 22-28°C. Surface growth of molds (filamentous fungi) and film-forming yeasts was prevented by ultraviolet lamps (Etchells et al., 1975). The pack-out ratio was 50% cucumbers and 50% brine by weight. Cucumbers were washed before brining only when excessive soil adhered to the fruit.

Naturally fermented cucumbers were acidified at brining with 0-0.16% acetic acid, based on total weight of cucumbers and brine. Brines were not inoculated, and fermentation was due to indigenous microflora. Inoculated fermentations were acidified at brining with 0-0.16% acetic acid, buffered with 0-0.30% anhydrous sodium acetate after ca 24 hr and inoculated with L. plantarum WSO, ca 10⁵ cells/ml, after buffer addition. Inoculated fermentations differed from the controlled fermentation process of Etchells et al. (1973, 1975) in that cucumbers were not washed nor sanitized with sodium hypochlorite. Purging gases were bottled air or N₂ regulated with flowmeters (Air Products and Chemicals Corporation, Allentown, PA) at flow rates of 5-100 ml/min-gal. Most brines were purged from the bottom using fritted glass spargers, porous plastic tubing (pore size 10-20 μ m; Porex Corporation, Fairburn, GA), or 3/16 inch, plastic tubing punctured several times with a 22-gauge needle. In one fermentation, purging was accomplished with the remote purging system shown in Fig. 1, such that air bubbles did not contact cucumbers.

Analyses

Pectate depolymerase (PD) activity of dialyzed brine was measured by % viscosity loss of ca 1% sodium polygalacturonate solution (SPG) after 20 hr at 30°C (Bell et al., 1955). Initial flow time was ca. 35 seconds in no. 300 Ostwald-Fenske viscosity pipettes. SPG was buffered at pH 5.0 with 0.35% citric acid monohydrate and 0.13% NaOH unless otherwise specified. For some tests, SPG was buffered at pH 9.0 with 0.76% NaB₄O₇·10 H₂O. Adjective ratings for PD activity, in relation to % viscosity loss, were: 0-9%, negative to weak; 10-19%, moderately active; 20-28%, strong; 29-37%, very strong; and >37%, extremely strong (Bell et al., 1955). Bell et al. originally interpreted viscosity loss to be due to polygalacturonase (PG) activity. Since that time the presence of a second group of pectin depolymerase enzymes has been reported, namely pectin transeliminases (lyases) (Rombouts and Pilnik, 1972). We did not attempt to distinguish between these two types of depolymerases in this work and so attribute viscosity loss to the more general "pectate depolymerase" activity.

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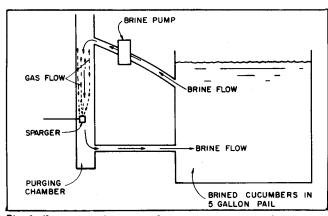


Fig. 1—Remote purging system for preventing contact of air bubbles with brined cucumbers. The purging chamber was made of 3.8 cm diameter, clear plastic pipe. Brine and air flowed countercurrently. No visual air bubbles entered the pail of cucumbers.

Cucumber firmness was measured with the Magness-Taylor fruit pressure tester (5/16 inch tip). Fermentations with average firmness of <3 lb were considered too soft for meaningful measurement and were not included in analyses of variance. Titratable acidity (pH 8.3 end point) and pH were measured with standard NaOH and a combination glass electrode.

Microbial isolates

Bacteria, yeasts, and molds were isolated from brine by pour and streak plate techniques, and from cucumbers by placing bits of cucumber skin (ca 1 cm², cut to a depth of ca 0.4 cm with a sterile knife) on agar plates. For cucumbers with skin blisters, sterile tweezers were used to place the skin covering the blisters onto agar plates.

Media included acidified dextrose agar (5 ml of 10% tartaric acid per 100 ml melted medium for yeasts and molds), trypticase soy agar + 1% glucose (total aerobic bacteria), and violet red bile agar + 1% glucose (total Enterobacteriaceae; Mossel et al., 1962). The isolated organisms were incubated at 30°C in cucumber juice broth (Fleming and Etchells, 1967) at various acetic acid, lactic acid, and NaCl concentrations. Pectate depolymerase activity of the isolates was measured as previously described.

Commercial fermentations

Air- and N₂-purged, size no. 3 (3.8-5.1 cm diam), pickling cucumbers brined in commercial-size (400-1,200 bu capacity) tanks were studied with the cooperation of local pickle companies, designated as Plant 1 and Plant 2. Acidification levels were 0, 0.05, and 0.16% acetic acid (200 grain vinegar was used as the acidulant) based on total volume of cucumbers and brine. Brines were purged within 1 day of brining at 20-30 ft³/hr (equivalent to 1.0-1.5 ml/ min-gal assuming a 1000-bu brining tank) with a side-arm apparatus similar to that described by Costilow et al. (1977). Nitrogen purging was continuous. Air purging was intermittent, with purging by day and no purging at night. NaCl concentrations of brines during fer-

Table 1-Air-purged, natural fermentations: a effect of acetic acid on cucumber firmness and PD activity

Acetic acid (%)	Inital brine pH	Firmness ^b (lb)	PD activity ^c (% visc. loss)	
Purged				
0.16	2.8	16.4	6	
0.12	2.9	9.1	45	
0.08	3.1	5.1	72	
0.04	3.3	<3	86	
0.00	6.2	<3	93	
Not purged				
0.00	6.2	16.1	9	

Table 2-Air-purged, inoculated fermentations: a effect of acetic acid and sodium acetate on cucumber firmness and PD activity

Acetic acid (%)	Na acetate (%)	Initial brine ^b pH	Firmness ^c (lb)	PD activity ^d (% visc. loss)
0.16	0.30	2.8	20.1	2
80.0	0.15	2.8 3.1	17.2	3
0.05	0.10	3.2	10.1	21
0.03	0.05	3.4	<3	60
0.00 ^e	0.00	7.2	<3	85

Air purging begun at brining at 5 ml/min-gal in 5-gal palls.

mentation were 6-7%. Cucumber and brine samples in 1-qt jars were transported to our laboratory in ice chests and refrigerated until the next day. Microbial isolates from brines and cucumbers were tested for PD activity, as previously described. From Plant 1, brines from 5 air- and 5 N2-purged fermentations were sampled. Sections of cucumber skin (3-4 sections/cucumber, ca 1 cm²/ section) were sampled from 12 cucumbers (2 per tank) taken from 4 air- and 2 N2-purged fermentations. From air-purged fermentations, the cucumber samples were chosen so that, of the 2 cucumbers from each tank, 1 had soft spots and/or skin blisters and the other did not. The cucumbers from the 2 N2-purged fermentations did not have soft spots or skin blisters. From Plant 2, 6 cucumbers from the nonacidified, air-purged, natural fermentations were examined for pectinolytic molds. Each cucumber had skin blisters and/or soft spots.

RESULTS

Laboratory fermentations

Addition of 0.16% acetic acid (equilibrated) prevented cucumber softening and development of pectate depolymerase (PD) activity in the brine of air-purged, natural fermentations (Table 1). Cucumbers from air-purged fermentations acidified with 0.16% acetic acid were comparable in firmness to cucumbers from nonpurged, nonacidified, natural fermentations that served as experimental controls. Fermentations containing 0.0-0.12% acetic acid had extensive softening and "very strong" brine PD activities. PD activity developed within 3 days after brining and approached maximum levels within 7 days (Fig. 2). Cucumbers from air-purged fermentations often had soft spots and blister-like skin lesions. Beneath the skin blisters were gas-filled cavities where underlying tissue had been dissolved.

In air-purged, inoculated fermentations, acidification with 0.16% acetic acid followed by buffering after ca 24 hr with 0.30% sodium acetate also prevented cucumber softening and development of PD activity (Table 2). These are the levels of acetic acid and sodium acetate suggested in the controlled fermentation process of Etchells et al. (1973). Acidification with 0.08% acetic acid and buffering with 0.15% sodium acetate prevented development of PD activity, but reduced firmness was found. Over 50% of the cucumbers from this fermentation had small soft spots and/or skin blisters. Extensive cucumber softening and "strong" brine PD activities were observed in fermentations acidified with 0.05% or less acetic acid. As with natural fermentations, PD activity in inoculated fermentations appeared within 3 days after brining (Fig. 3).

Delay of air purging, until growth of indigenous lactic microflora had reduced the brine pH to 4.0, prevented cucumber softening and development of PD activity in nonacidified, natural fermentations (Table 3). Cucumber softening and PD activity did not develop even at the extremely high aeration rate of 100 ml/min-gal. Cucumbers from these fermentations (nos. 3 and 4) did not have soft spots or skin blisters and were comparable in firmness to cucumbers from a nonpurged, nonacidified, natural fermentation (no. 5) and air- and N2-purged, acidified, buffered, inoculated fermentations (nos. 6 and 7). When air purging was begun immediately after brining (nos. 1 and 2), the cucumbers became very soft, and "extremely strong" PD activities developed in the brines.

Firmness of cucumbers from air-purged fermentations acidified with 0.16% acetic acid, buffered with 0.30% sodium acetate, and inoculated with L. plantarum compared favorably with similarly treated N2-purged fermentations (Table 4). As before, at even the extremely high aeration rate of 100 ml/min-gal, softening and PD activity were prevented. Skin blisters and soft spots were not present.

a Air purging was begun at brining at 100 mi/min-gal in 1-qt jars.

Number 2 size cucumbers (2.7—3.8 cm dlam) were used.

b LSD_{.05} = 3.4 lb; duplicate jars were tested 14 days after brining,

5—8 cucumbers per jar.

c PD activity 7 days after brining.

b Brine pH after acidification, Na acetate buffer was added ca. 24 hr later.

c 24 nr later. C LSD_05 = 2.9 lb; 20 cucumbers per pail tested 10 days after brining. d PD activity 7 days after brining.

Microbial isolates (38) obtained from this fermentation were studied for PD activity.

Purging of brines so that air bubbles were not in direct contact with cucumbers, by the system illustrated in Fig. 1, did not prevent softening nor PD development in the absence of adequate acidification (Table 5). Thus, direct contact of air bubbles and cucumbers was not a prerequisite for subsurface mold growth.

Commercial fermentations

In commercial fermentations acidified with 0.05% acetic acid, N2-purged cucumbers were significantly firmer (P ≤ 0.05) than air-purged cucumbers (Table 6, Plant 1). Softening was evidenced by the presence of soft spots and skin blisters (Fig. 4). PD-producing molds were isolated from airpurged cucumbers having soft spots and/or skin blisters. Samples of skin from cucumbers that had been air purged but did not have soft spots or blisters did not yield molds. Skin samples from N₂-purged cucumbers did not yield molds. Also, no molds were found among microbial isolates obtained from the brines of air- and N2-purged fermentations. Of the 22 molds isolated, all produced PD when grown in cucumber juice broth with 5% NaCl. Of the 59 yeasts isolated, none produced PD activity. PD activity was

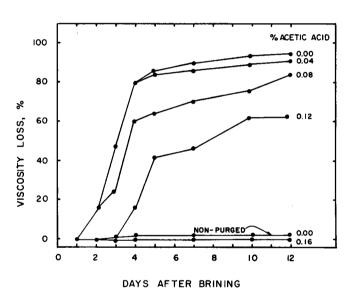


Fig. 2-Development of PD activity in air-purged, acidified, natural fermentations.

not present in the N2-purged brines or in 4 of the 5 airpurged fermentations. PD activity was "moderate" in 1 air-purged brine. The cucumbers sampled at Plant 1 were taken within 60 cm depth of a hole cut in the brine tank headboards.

At Plant 2, headboards were removed and cucumbers were sampled from the top 60 cm of the fermentation tanks and from the middle of the tanks ca. 1.5 m down from the surface. Cucumbers sampled from the tops of tanks from acidified fermentations (0.16% acetic acid), including both air- and N2-purged, were significantly firmer $(P \le 0.05)$ than those from an air-purged, nonacidified, natural fermentation (Table 6, Plant 2). As before, softening in the nonacidified, air-purged fermentation was evidenced by small skin blisters and soft spots, from which PD-producing molds were isolated. Cucumbers sampled from the middle of the tanks were not significantly reduced in firmness, and these cucumbers were nearly free of soft spots and skin blisters. PD activity of these brines was "negative to weak." -Continued on next page

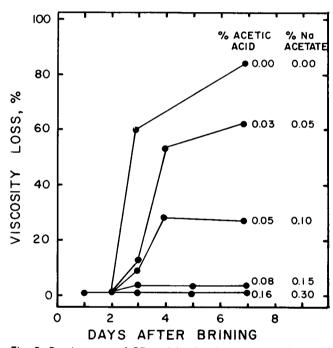


Fig. 3—Development of PD activity in air-purged, acidified, buffered, inoculated fermentations.

Table 3—Effect of time of initiating air purging on cucumber softening

	Purging st	arted	Pu	ırging		
No.ª	Days after brining	Brine pH	Gas	Rate, ml/min-gal	Firmness ^d (lb)	PD activity ^e (% visc. loss)
			Natural ferment	tations ^b		
1	o [®]	6.3	Air	5	<3	85
2	0	6.1	Air	100	<3	89
3	4	4.0	Air	5	18,5	4
4	4	4.0	Air	100	19.9	3
5	- •	-	Not purged		19.9	2
			Acidified, inoculated f	ermentations ^c		
6	0	3.0	Air		20.2	3
7	0	3.0	N ₂	5	18.4	4

Numbers 2, 4, and 5 were 1-gal jars; nos 1, 3, 6, and 7 were 5-gal pails. Natural fermentations were not acidified nor inoculated.

Acidified (0.16% acetic acid), buffered (0.30% Na acetate), and inoculated. d LSD 05 = 3.3 lb; 10 cucumbers per treatment tested 27 days after brining. PD activity 14 days after brining.

Microbial isolates

Three days after brining, 38 microbial isolates (see footnote e, Table 2) were collected from an air-purged, nonacidified, natural fermentation. At this time, PD activity in the brine was rapidly increasing. Cucumbers in this fermentation subsequently became soft. The 38 isolates included 30 bacteria (11 were Enterobacteriaceae), 4 yeasts, and 4 molds. Only the 4 molds produced PD activity. None of the 38 isolates produced PD activity in pH 9.0 SPG. One mold was identified as a Geotrichum species and three as members of the order Mucorales, according to descriptions of Ainsworth et al. (1973).

In cucumber juice broth, at ca pH 3.0-4.5, acetic acid was more effective than lactic acid in preventing growth of the 4 mold isolates (Table 7). Acetic acid, 0.3%, ca. pH 4.0, prevented growth of the isolates if at least 5.3% NaCl was present; But two isolates grew when only 2.6% NaCl was present. In contrast, lactic acid, 0.9%, ca pH 3.0, and 5.3% NaCl did not prevent growth of the mold isolates. Growth was prevented by 0.9% lactic acid at NaCl levels of 6.6 and 7.9%. However, 0.4% lactic acid, pH 3.4, did not prevent growth of the mold isolates at NaCl levels of 2.6.

Table 4-Comparison of firmness and PD activity in air- and N2purged, acidified, buffered, inoculated cucumber fermentations^a

Purge gas	Purge rate (ml/min-gal)	Firmness ^b (lbs)	PD activity ^C (% visc. loss)		
Air	5	20.3	1		
Air	100	21.0	7		
N ₂	5	21.8	2		

^a Brines at equilibration contained 0.16% acetic acid, 0.30% Na acetate. Purging was begun at brining in 5-gal pails.

c PD activity 12 days after brining.

Table 5-Effect of remote air purging on firmness and PD activity in naturally fermented cucumbers

Purge gas ^a	Type of purging	Firmness ^b (lb)	PD activity ^c (% visc. loss)		
Air	Remote	11.8	78		
Air	Bottom	11.1	65		
N ₂ (control)	Bottom	19.0	2		

Air purging begun at brining at 5 ml/min-gal in 5-gal pails. LSD_{.05} = 2.8 lb; 20 cucumbers per treatment tested 13 days after brining.

c PD activity 9 days after brining.

Table 6-Effect of purging gas and acidification on cucumber firmness in commercial fermentations

No. of tanks	_	Acetic	Firm	ness, Ib ^a	Soft spots,% ^b		
	Purge gas	acid (%)	Тор	Middle	Тор	Middle	
Plant 1							
5	Air	0.05	15.9	ND	+	ND	
5	N_2	0.05	19.1	ND	_	ND	
Plant 2			-				
1	Air	0.00	18.4	20.1	24	0	
2	Air	0.16	21.2	21,3	1	2	
4	N ₂	0.16	20.6	21.3	0	Ō	

Top = cucumbers from within 60 cm of headboards; middle = cucumbers taken from ca 1.5m down from the surface with a fish net. LSD 05 = 2.0 lb for Plant 1 and 2.1 lb for Plant 2; 20 cucumbers per tank tested; ND = not determined.

b 50 cucumbers per tank tested; only presence (+) or absence (-)

of soft spots noted at Plant 1.

5.3, and 6.6%, and 3 of the 4 isolates grew when the NaCl level was 7.9%.

The pH of inoculated broths that remained free of visual growth was unchanged after incubation at 30°C for 12 days. The pH of broths showing growth had increased to beteeen 6.5 and 8.1, indicating that the molds had metabolized and/or neutralized the added acetic and lactic acids. PD activity was present in broths showing mold growth.

DISCUSSION

THE CONSISTENT ABSENCE of cucumber softening in air-purged, controlled fermentations (acidified, buffered and inoculated; Etchells et al., 1973) that we have observed over a period of several years can likely be attributed to acidification with acetic acid and to rapid growth of the added lactic culture. We found that the addition of 0.16% acetic acid (equilibrated) to air-purged fermentations was effective in preventing mold-induced softening, even at air flow rates greatly in excess of those needed to prevent bloater damage. This finding contrasted greatly with the consistent occurrence of softening at similar air flow rates in laboratory studies when brines were not initially acidified, or were acidified at low levels of acetic acid. Gates and Costilow (1981) also found cucumber softening in laboratory-size fermentations purged at high rates of air. Their brines did not exceed 0.06% acetic acid.

Our laboratory fermentations were 50% cucumbers and 50% brine. Commercial fermentations may be as much as 70% cucumbers due to weight compaction. The addition

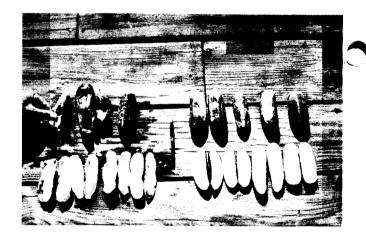




Fig. 4-(Top) Left, air-purged cucumbers from Plant 1. Cucumbers were taken within 60 cm of the headboards. Note hollow spots and extensive softening. Right, N2-purged cucumbers from Plant 1. Cucumbers were firm. See Table 6 for related information. (Bottom) Skin blisters on cucumbers from air-purged fermentations at Plant 1. The tissue was soft underneath the blisters, while other parts of the cucumbers were firm.

LSD 05 = 2.5 lb; 10 cucumbers per treatment tested 13 days after brining.

Table 7—Growth of mold isolates in cucumber juice broth in the presence of acetic acid, lactic acid, and NaCla

	Acetic acid						Lactic acid					
√% NaCl	рН	%	M1	M2	М3	G1	pН	%	M1	M2	М3	G1
2.6	4.5	0.1	+	+	+	+	4.4	0.1	+	+	+	+
	4.0	0.3	_	+	_	+	3.9	0.2	+	+	+	+
	3.5	1,2	_		_	_	3.4	0.4	+	+	+	+
	3.0	4.3		_	_		3.0	0.9	+	+	+	+
5.3	4.4	0.1	+	+	+	+	4.3	0.1	+	+	+	+
	4.0	0.3	_		_	_	3.9	0.2	+	+	+	+
	3.4	1.2		_	_	_	3.4	0.4	+	+	+	+
	3.0	4.3	_	_	_	_	2.9	0.9	+	+	+	+
6,6	4.4	0.1	+	+	+	+	4.3	0.1	+	+	+	+
	3.9	0.3		_	_		3.9	0.2	+	+	+	+
	3.4	1.2	_	_	_	_	3.4	0.4	+	+	+	+
	3.0	4.3	_	_	_		2.9	0.9	_	_	_	-
7.9	4.3	0.1	+	+	+	_	4.3	0.1	+	+	+	+
	3.9	0.3		_	_	_	3.8	0.2	+	+	+	_
	3.4	1.2	-	-	-	_	3.4	0.4	+	+	+	_
	2.9	4.3	_			_	2.9	0.9	_	_	-	_

M1, M2, and M3 were identified as members of the order Mucorales and G1 was identified as a Geotrichum species. Test tubes were incubated at 30°C and growth determined at 3 and 10 days (no difference found); + = growth, - = no growth.

of 0.16% acetic acid based on total fermentation volume leads to initial concentrations in the brine of 0.32%, assuming 50% cucumbers, to as high as 0.53% acetic acid, assuming 70% cucumbers. Thus, during the critical first few days after brining, before growth of lactic acid bacteria, brine concentrations will exceed the equilibrium acetic acid level of 0.16%.

Levine and Fellers (1940) found that 0.27% acetic acid in glucose broth, pH 4.1, inhibited growth of Aspergillus niger and that 0.59% acetic acid, pH 3.9, was toxic and killed both mold spores and mycelium. Kirby et al. (1937) reported that 0.2% acetic acid in liquid culture at pH 3.5 prevented growth of Aspergillus flavus, A. fumigatus, A. niger, A. oryzae, Neurospora sitophila, Penicillum expansum, P. leuteum-purpurogenum, and Rhizopus nigricans. Costilow et al. (1980) identified molds isolated from softened air-purged cucumbers as species belonging to the genera Alternaria, Fusarium, and Mucor.

Prevention of mold-induced softening by delaying air purging until the growth of lactic acid bacteria had reduced the brine pH to 4.0 can not be explained by lactic acid as the sole inhibitor of mold growth since mold isolates grew in the presence of 0.4% lactic acid and 7.9% NaCl at pH 3.5. Furthermore, Kirby et al. (1937) found that 0.6% lactic acid was not inhibitory to the previously mentioned 8 mold species. Levine and Fellers (1940) also found that 3.08% lactic acid in glucose broth (no NaCl), pH 2.5, did not prevent growth of A. niger.

Gates and Costilow (1981) reported that softening was prevented by delaying air purging for 2 days or more. Furthermore, they found that addition of 0.035% potassium sorbate prevented mold-induced cucumber softening in airpurged fermentations. Thus, addition of acetic acid, sorbic acid, or both to cucumber cover brines may be a practical means for reducing the possibility of softening in air-purged fermentations.

Whether air should be considered safe for use by commercial briners as a purging gas remains questionable, however, even when acetic acid and/or sorbic acid are present. Possibilities exist for development of acetic and sorbic acid-resistant molds and yeasts that are pectinolytic and for development of off flavors and off colors due to oxidation and/or excessive growth of aerobic microorganisms that may be stimulated by dissolved O₂ present in air-purged brines. Some briners, aware of the potential hazards of air purging, employ various purging regimes to lower the probability of serious problems. Some air-purge intermittently,

and others purge with N₂ for the first few days until the fermentation is underway and then purge with air. In any case, air purging requires careful controls and brine monitoring to prevent softening. In contrast, N₂ purging is free of the hazards of chemical and biological spoilage and has a favorable cost:benefit ratio when compared to unpurged fermentations.

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